

CO₂, CH₄ and N₂O flux changes in degraded grassland soil of Inner Mongolia, China

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Abstract: The main purpose of this study was to explore the dynamic changes of greenhouse gas (GHG) from grasslands under different degradation levels during the growing seasons of Inner Mongolia, China. Grassland degradation is associated with the dynamics of GHG fluxes, e.g., CO₂, CH₄ and N₂O fluxes. As one of the global ecological environmental problems, grassland degradation has changed the vegetation productivity as well as the accumulation and decomposition rates of soil organic matter and thus will influence the carbon and nitrogen cycles of ecosystems, which will affect the GHG fluxes between grassland ecosystems and the atmosphere. Therefore, it is necessary to explore how the exchanges of CO₂, CH₄ and N₂O fluxes between soil and atmosphere are influenced by the grassland degradation. We measured the fluxes of CO₂, CH₄ and N₂O in lightly degraded, moderately degraded and severely degraded grasslands in Inner Mongolia of China during the growing seasons from July to September in 2013 and 2014. The typical semi-arid grassland of Inner Mongolia plays a role as the source of atmospheric CO₂ and N₂O and the sink for CH₄. Compared with CO₂ fluxes, N₂O and CH₄ fluxes were relatively low. The exchange of CO₂, N₂O and CH₄ fluxes between the grassland soil and the atmosphere may exclusively depend on the net exchange rate of CO₂ in semi-arid grasslands. The greenhouse gases showed a clear seasonal pattern, with the CO₂ fluxes of −33.63–386.36 mg/(m·h), CH₄ uptake fluxes of 0.113–0.023 mg/(m·h) and N₂O fluxes of −1.68–19.90 μg/(m·h). Grassland degradation significantly influenced CH₄ uptake but had no significant influence on CO₂ and N₂O emissions. Soil moisture and temperature were positively correlated with CO₂ emissions but had no significant effect on N₂O fluxes. Soil moisture may be the primary driving factor for CH₄ uptake. The research results can be in help to better understand the impact of grassland degradation on the ecological environment.

Keywords: grassland degradation; semi-arid grassland; greenhouse gases; CO₂; CH₄; N₂O; Inner Mongolia

1 Introduction

Carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) are the three most important greenhouse gases (GHGs) that contribute to global climate change (Lang et al., 2011). The global warming potentials of CH₄ and N₂O are approximately 28 and 265 times of CO₂ (Rowlings et al.,

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2012; Li et al., 2015; Yang et al., 2015). With the significant increase in greenhouse gas (GHG) concentrations, global climate change intensifies and most of climate warming has been attributed to human activities (Lavoie et al., 2013; Marín-Muñiz et al., 2015), such as deforestation, farming practices and fuel combustion (Tang et al., 2006; Li et al., 2015). GHG can be produced and absorbed through related processes in soils (Liu et al., 2008) and are also closely related to terrestrial ecosystem carbon (C) and nitrogen (N) cycling (IPCC, 2007).

As an important part of terrestrial ecosystem, grassland covers approximately 1/4 to 1/3 of the land surface of the Earth and is an important component of nutrient cycling in the system. Human activities and climate change have a serious impact on nutrient cycling (Bontti et al., 2009). The changes in the amount of GHG exchange between the atmosphere and grassland ecosystems may also have a significant impact on global climate change (Norman et al., 1992; Lal, 1999). As one of the global ecological environmental problems, the area of grassland degradation has reached more than 49% of the global grassland area (Gang et al., 2014), which has changed the vegetation productivity as well as the accumulation and decomposition rates of soil organic matter and thus will influence the C and N cycles of ecosystems (Kimble et al., 2013), which will affect the GHG fluxes between grassland ecosystems and the atmosphere. Therefore, it is necessary to study the exchange of CO₂, CH₄ and N₂O fluxes between soil and atmosphere influenced by grassland degradation.

Grazing acts as one of the main causes of grassland degradation according to the studies (Wiesmeier et al., 2009; Wu et al., 2014) on the influence of different grazing intensities on grassland soil nutrients and physicochemical properties. Most studies focus on the impact of grazing on GHG fluxes and show that heavy grazing may reduce the potential of steppe soils as a source or sink of atmospheric CH₄ and N₂O (Wolf et al., 2010; Chen et al., 2011a). Some researches show that grazing can increase N₂O emissions (Flechard et al., 2007; Abdalla et al., 2009; Rafique et al., 2012). Reducing grazing pressure in short term may not be able to increase the potential of managed grasslands as a sink for GHGs (Allard et al., 2007). Meanwhile, GHG (CO₂, CH₄ and N₂O) fluxes in grasslands are also sensitive to environmental conditions, such as soil moisture (Saggar et al., 2007; Xu et al., 2008; Luo et al., 2013) and the temperature of soil top layer (Horváth et al., 2010; Abalos et al., 2014). Grassland degradation is a result of the combined effects of many factors including human activities, management systems and climatic conditions.

The area of natural grasslands account for approximately 41% of the national land area in China (Wang et al., 2005). In northern China, 78% of the grassland area is the semi-arid temperate grasslands (Chen and Wang, 2000). Reports on the state of the environment in China issued in 2011 showed that 90% of natural grasslands have experienced different degrees of degradation (Ministry of Environmental Protection the People's Republic of China, 2011). However, few reports are available on soil-atmospheric GHG fluxes in grassland with different degrees of degradation. So we measured the GHG fluxes of the degraded grasslands dominated by *Stipa grandis* in the Xilin River Basin of Inner Mongolia, China. The objectives of this study were (1) to investigate the temporal variations of CO₂, CH₄ and N₂O fluxes during the growing seasons of 2013 and 2014 in different degraded semi-arid grasslands of Inner Mongolian China; (2) to examine the effects of grassland degradation on CO₂, CH₄ and N₂O fluxes; and (3) to assess the effects of environmental regulating factors (soil temperature and moisture) on the fluxes of CO₂, CH₄ and N₂O.

2 Materials and methods

2.1 Study area

The study area lies in the Xilin River Basin of Inner Mongolia Autonomous Region, China (44°10'N, 116°22'E). The area has a temperate terrestrial monsoon climate, with a cold and dry winter and a warm and wet summer. The annual mean temperature is 2.6°C, with monthly mean temperatures ranging from -18.8°C in January to 21.2°C in August. The mean annual precipitation is 340 mm, with 80%–90% falling during the growing season of May to September.

The dominant plant species is *S. grandis*; however, in severely degraded areas, the dominant species is *Stipa krylovii*. The soil is classified as a mollisols (USDA soil taxonomy). The area has been continuously grazed during the growing season since 1956. Grassland communities vary with grazing intensity. The areas near residential or livestock water sources are most obviously impacted. Grazing pressure and degradation levels vary along the radial direction of grassland communities in these areas (Chen and Wang, 2000). We chose a representative herdsman's place of residence in the study area that provided land with a range of degradation levels to establish three linear transects (A, B, and C) in a radial pattern (Fig. 1).

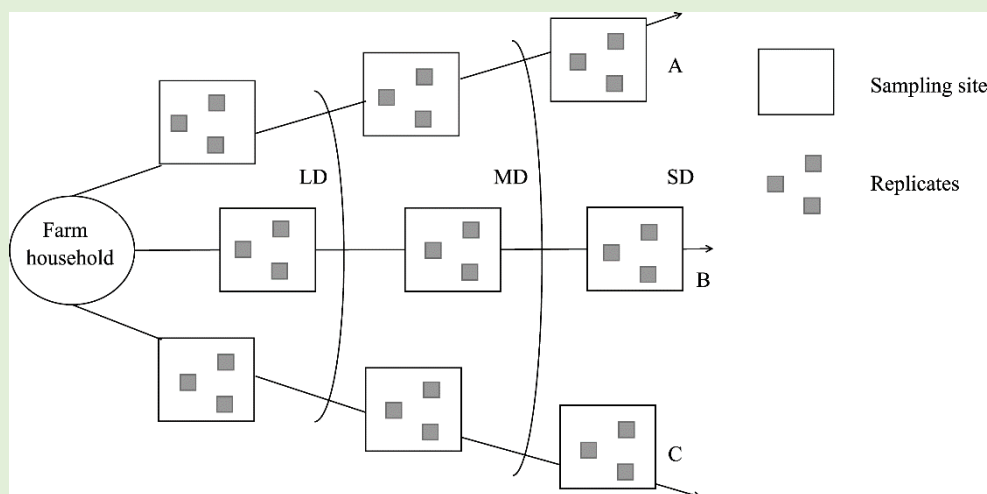


Fig. 1 Distribution of sampling sites. A, B, and C represent three transects. LD, light degradation; MD, moderate degradation; SD, severe degradation.

2.2 Determination of the grassland degradation level

The length of transects ranged from 1500 to 2000 m. Each transect of grassland was classified into three stages of degradation as light (LD), moderate (MD), and severe (SD) degradation (Table 1). The basis and method of classification referred to An et al. (1999) and Wen et al. (2016).

Table 1 Species and vegetation cover of grasslands in different degradation levels

Degree of degradation	Species	Vegetation cover (%)
LD	<i>Stipa grandis</i> + <i>Chenopodium album</i> + <i>Cleistogenes squarrosa</i> + <i>Stipa krylovii</i>	26.73
MD	<i>S. krylovii</i> + <i>S. grandis</i> + <i>C. album</i> + <i>C. squarrosa</i>	23.82
SD	<i>S. krylovii</i> + <i>C. album</i> + <i>Euphorbia humifusa</i> + <i>Agropyron michnoi</i>	30.19

Note: LD, light degradation; MD, moderate degradation; SD, severe degradation.

2.3 Sampling

Three replicates (Fig. 1) were randomly collected at each sampling site to ensure the aboveground vegetation, soil conditions can be best represented. Fluxes of CO₂, CH₄ and N₂O were measured using static chambers and gas chromatography from June to September in 2013 and 2014, respectively (Wang and Wang, 2003). The static chamber was made of 8-mm-thick black poly-methyl methacrylate with internal and external reflective films on frame of stainless steel inserted into the soil at a depth of 5 cm with a water groove to make the static chamber airtight. The length, width and height of each chamber are 40, 40 and 30 cm, respectively. There was a fan (10-cm diameter) installed on the top wall of each static chamber to mix the air in the closed static chamber, with a 12V battery for its power supply. Before placing the chambers on the frame, the grass within the frame was cut to the ground. The flux measurements were taken twice every month in the growing seasons from 09:00 to 11:00 LST in the morning at 10-min intervals. Gas samples (60 mL each) were collected with 100-mL plastic syringes at fixed intervals of 0, 10, 20 and 30 min after closure and stored in gas sampling bags. The gas samples were analyzed by a

modified gas chromatograph (Agilent 7890A, Agilent Technologies, USA) to obtain CO₂, CH₄ and N₂O concentrations. Fluxes of CO₂, CH₄ and N₂O were determined from the slope of the mixing ratio change with four sequential samples. Air temperature, temperature in the static chambers, soil temperature and moisture at the soil depth of 0–10 cm were monitored while gas samples were measured. The fluxes were calculated according to the following equation:

$$F = \rho \times \frac{V}{A} \times \frac{\Delta C}{\Delta A}, \quad (1)$$

where F is the flux (μg/(m·h)) of GHG; ρ is the density (mol/m³) of GHG; $\Delta C/\Delta T$ is the slope of the linear regression for gas concentration gradient through time; A and V are volume (m³) and the static chamber base area (m²), respectively.

2.4 Soil properties analysis

Soil of the three replicate samples from each sampling site was collected in June 2013 at the soil depth of 0–10 cm using an 8-cm diameter cylindrical soil sampler. The three soil samples were combined to produce a single composite sample. Soil bulk density and soil water content were measured referred to Li et al. (2015). Soil pH was determined in suspensions composed of a 1:5 ratio of soil to water using a PHS-3S pH meter. Soil organic matter, total N, NH₄⁺-N and NO₃⁻-N were measured referred to Wen et al. (2016).

2.5 Statistical analyses

CO₂, CH₄ and N₂O fluxes, soil temperature, moisture, pH values and organic matter, total N, NH₄⁺-N and NO₃⁻-N for each degree of degradation were calculated by averaging the samples from the three sites. All variables were checked for normal distribution and homogeneity for variance before analysis. One-way analyses of variance were performed to examine the differences in GHG fluxes at different dates and degrees of degradation (least significant difference; $P < 0.05$). The significance of the impacts of date, degradation, year and their interaction effective on GHG fluxes, soil temperature, and soil water content was assessed using Multi-factor analysis of variance. Pearson's correlations was used to determine the relationships between environmental factors, soil properties and GHG fluxes during the growing season. SPSS 20.0 was used for all data analyses.

3 Results

3.1 Soil properties

Soil organic matter and total N at the LD site were higher than at the MD and SD sites, whereas the bulk density was the highest at the SD site; however, there were no significant differences between different sites. The average soil temperature and soil water content during the growing season was the highest at the MD site and soil pH was the lowest at the LD site. The concentration of NO₃⁻-N decreased in the order of LD>SD>MD, and the concentration of NO₃⁻-N at the LD site were significantly higher than at MD site, whereas the concentration of NH₄⁺-N increased with the increase of degradation level (Table 2).

Table 2 Soil properties of grasslands under different degradation level

Property	LD	MD	SD
Soil organic matter (g/kg)	26.24±2.24	24.48±2.04	22.88±5.70
Total N (g/kg)	1.47±0.01	1.37±0.01	1.22±0.03
Bulk density (g/cm ³)	1.28±0.02	1.34±0.09	1.44±0.11
Soil temperature (°C)	19.44±3.36	19.48±3.84	18.77±3.59
Soil moisture (%)	7.34±3.45	7.45±2.91	7.43±3.18
Soil pH	7.65±0.01	7.70±0.07	7.70±0.10
NO ₃ ⁻ -N (mg/kg)	9.94±0.94 ^a	6.40±1.33 ^b	8.61±2.54 ^{ab}
NH ₄ ⁺ -N (mg/kg)	0.11±0.18	0.48±0.42	0.63±0.55

Note: Different lowercase letters represent statistically significant differences among different degree of degradation at $P < 0.05$ level. Mean±SE, $n=9$.

3.2 Changes of CO₂ fluxes

CO₂ fluxes exhibited a single-peak pattern during the growing season in 2013 (Fig. 2a), with the minimum and maximum occurred on 22 September 2013 and 23 July 2013, respectively. In late July, CO₂ fluxes at the MD site was higher than those at the LD and SD sites. CO₂ absorption occurred at the SD site in late September. During the growing season, CO₂ fluxes in July was significantly higher than those in other months. Soil CO₂ fluxes fluctuated from -33.63 to 385.35 mg/(m·h), with averages of 193.89 (±15.59), 185.65 (±32.77) and 171.84 (±48.69) mg/(m·h) at the LD, MD and SD sites (Table 3), respectively. There was no significant difference among the different sites (Fig. 2b).

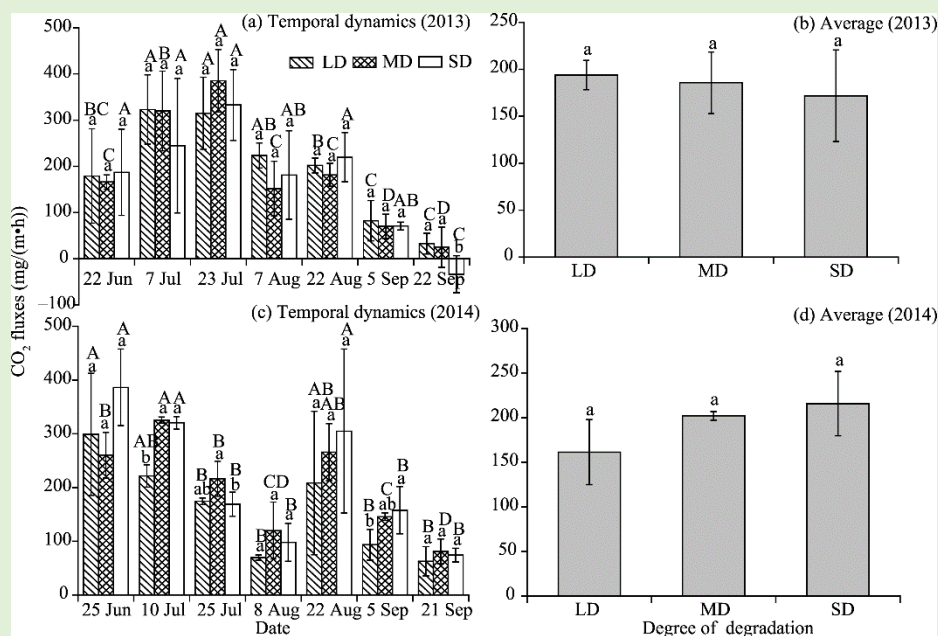


Fig. 2 CO₂ emissions from grassland under different degradation levels during the growing seasons of 2013 (a, b) and 2014 (c, d). The lowercase letters indicate statistically significant differences within the same date among the different degradation levels. The capital letters indicate statistically significant differences among the different dates under the same degradation levels. Error bars mean standard errors. LD, light degradation; MD, moderate degradation; SD, severe degradation.

Table 3 CO₂, CH₄, and N₂O fluxes at different degradation levels in grassland during the growing seasons from July to September in 2013 and 2014

Greenhouse gas	Degree of degradation	2013	2014	Average
CO ₂ (mg/(m·h))	LD	193.89±15.59	161.30±36.41	177.59±26.00
	MD	185.65±32.77	202.02±4.74	193.84±18.76
	SD	171.84±48.69	215.83±36.04	193.84±42.36
CH ₄ (mg/(m·h))	LD	-0.071±0.003 ^{ab}	-0.063±0.005 ^{ab}	-0.067±0.004 ^{ab}
	MD	-0.080±0.001 ^a	-0.073±0.005 ^a	-0.076±0.003 ^a
	SD	-0.066±0.001 ^b	-0.060±0.007 ^b	-0.063±0.008 ^b
N ₂ O (μg/(m·h))	LD	4.76±1.77	8.44±7.98	6.60±3.37
	MD	7.09±0.24	6.95±2.51	7.02±1.38
	SD	5.22±3.21	6.52±4.24	5.87±3.73

Note: Mean±SE, *n*=9. Different lowercase letters represent statistically significant differences among different degree of degradation at *P*<0.05 level.

In 2014, soil CO₂ fluxes exhibited a double-peak and single-trough pattern; the maximum occurred in late June at the SD site (386.36±71.31 mg/(m·h)), and the minimum occurred in late September at the LD site (74.29±12.79 mg/(m·h)). The soil CO₂ fluxes in late June, early July and

late August were significantly higher than those at other sampling dates during the growing season (Fig. 2c). The average flux during the growing season decreased in the opposite order for 2013 ($SD (215.83 \pm 36.41 \text{ mg}/(\text{m}\cdot\text{h})) > MD (202.02 \pm 4.74 \text{ mg}/(\text{m}\cdot\text{h})) > LD (161.30 \pm 36.41 \text{ mg}/(\text{m}\cdot\text{h}))$), and there was no significant differences among the different sites (Fig. 2d).

3.3 Changes of CH_4 fluxes

CH_4 fluxes for the grassland at the different degradation levels were negative in both 2013 and 2014 during the growing seasons. The soil absorbed CH_4 from the atmosphere (Fig. 3). The absorbed values of CH_4 first decreased, then increased and then decreased again in 2013. The maximum values of CH_4 uptake occurred in August 2014 ($-0.114 \text{ mg}/(\text{m}\cdot\text{h})$). The values at the LD and MD sites were significantly higher than those at the SD site (Fig. 3a). The average values of CH_4 uptake were ordered as $MD > LD > SD$ and the uptake of CH_4 at the MD site was significantly higher than at the SD site (Fig. 3b). The CH_4 fluxes showed the opposite trend in 2014 and the minimum values of CH_4 uptake occurred in August (Fig. 3c). In early September, the values at the MD site were significantly higher than those at the SD site. In other sampling dates, there were no significant differences between degraded grasslands. In 2014, the trend of average values of CH_4 uptake was same as in 2013 (Fig. 3d).

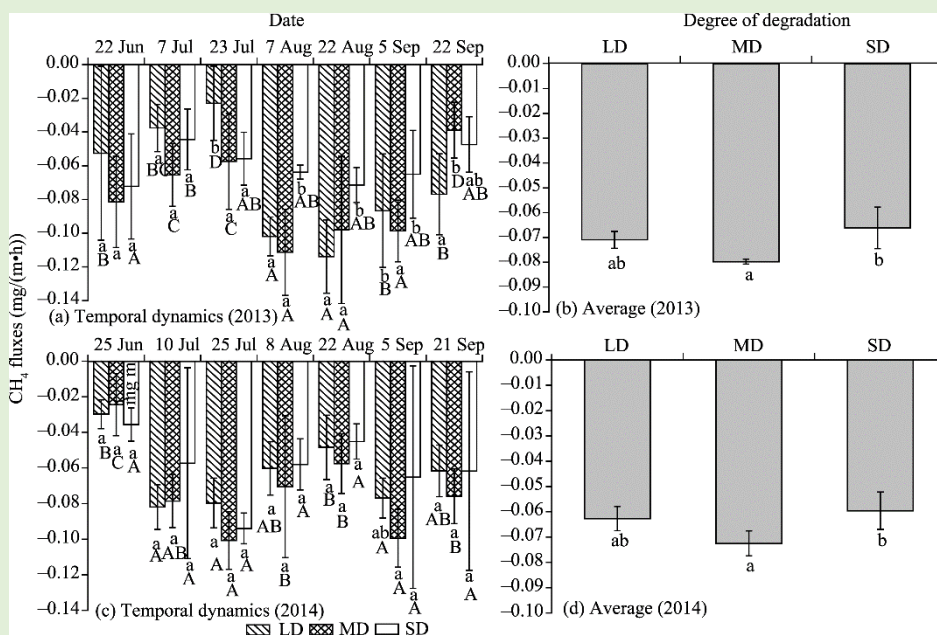


Fig. 3 CH_4 fluxes from grassland under different degradation levels during the growing seasons. The lowercase letters indicate statistically significant differences within the same date among the different degradation levels. The capital letters indicate statistically significant differences among the dates under the same degradation levels. Error bars mean standard errors.

3.4 Changes of N_2O fluxes

In 2013, the N_2O fluxes at the MD and SD sites showed the same trend of decrease followed by increase, whereas at the LD site the trend in N_2O fluxes was sinusoidal and there was a negative value appeared on 22 August 2013. N_2O emissions were higher in June and July but relatively lower in August and September. There were no significant differences between degraded grasslands in June, July and September (Fig. 4a). During the growing season, the average fluxes at the MD ($7.09 \mu\text{g}/(\text{m}\cdot\text{h})$) site was higher than at the SD ($5.22 \mu\text{g}/(\text{m}\cdot\text{h})$) and LD ($4.76 \mu\text{g}/(\text{m}\cdot\text{h})$) sites without significant difference (Fig. 4b). In 2014, the N_2O fluxes at the LD and SD sites showed the same trend of decrease followed by increase then decrease, whereas the fluxes showed a fluctuating downward trend at the MD site. In late July and late September, emissions of nitrous oxide were relatively lower (Fig. 4c); the average fluxes of N_2O were ordered $MD > LD > SD$, and there were no significant differences among the different degraded grasslands (Fig. 4d).

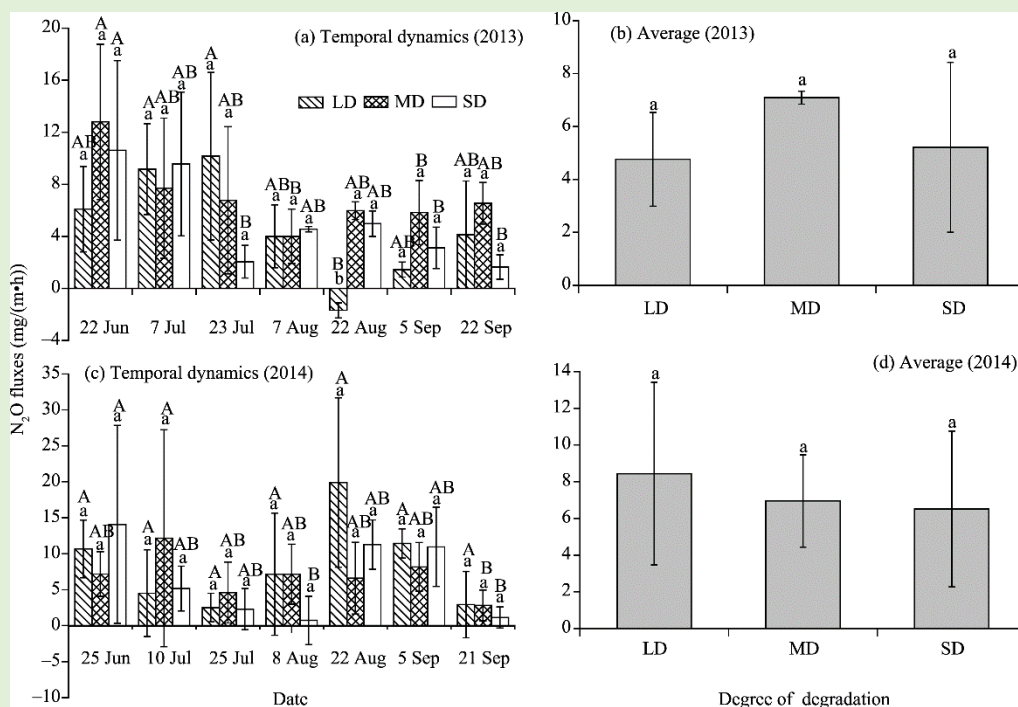


Fig. 4 N₂O emissions from grassland under different degradations during the growing seasons. The lowercase letters indicate statistically significant differences within same date among the different degradation levels. The capital letters indicate statistically significant differences among dates under the same degradation levels. Error bars mean standard errors.

3.5 Impacting factors of GHG fluxes

Changes in soil moisture and temperature during the growing season in 2013 and 2014 were shown in Figure 5. Correlation analysis showed that CO₂ emissions from different degraded grasslands were positively correlated with soil temperature, soil water content and temperature in static chamber (Fig. 6). The CH₄ fluxes was positively correlated with soil water content but negatively

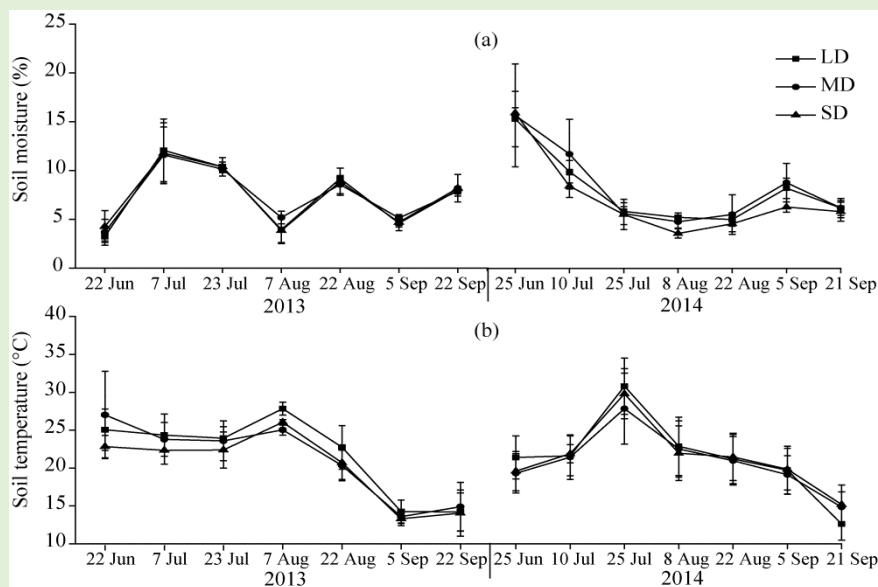


Fig. 5 Top soil (0–10 cm) moisture (a) and temperature (b) in 2013 and 2014

correlated with temperature in the static chamber. Additionally, no significant relationship was observed between CH_4 and soil temperature (Fig. 7). N_2O was significantly and positively related to litter total C content, whereas the N_2O fluxes had no significant correlations with soil temperature, soil water content and temperature in the static chamber (Fig. 8). Meanwhile, there were no significant relationship between GHG fluxes and NO_3^- -N. So did NH_4^+ -N, soil organic matter, total N, bulk density and pH.

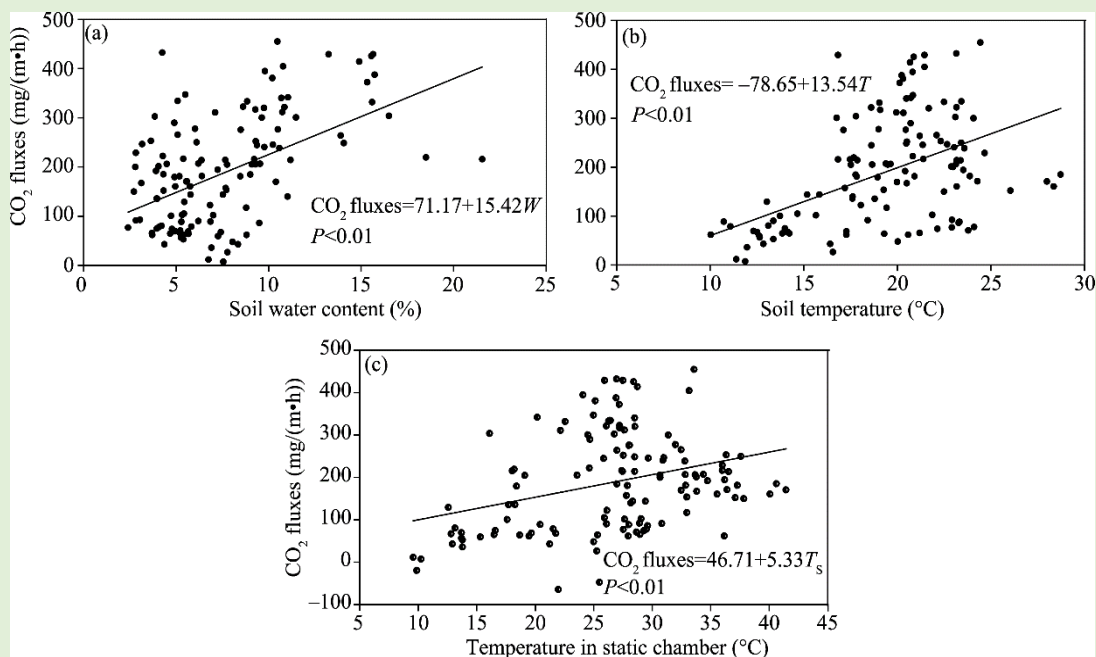


Fig. 6 Relationships of CO_2 fluxes with (a) soil water content (W), (b) soil temperature (T) at the surface soil (0–10 cm) and (c) air temperature in the static chamber (T_s)

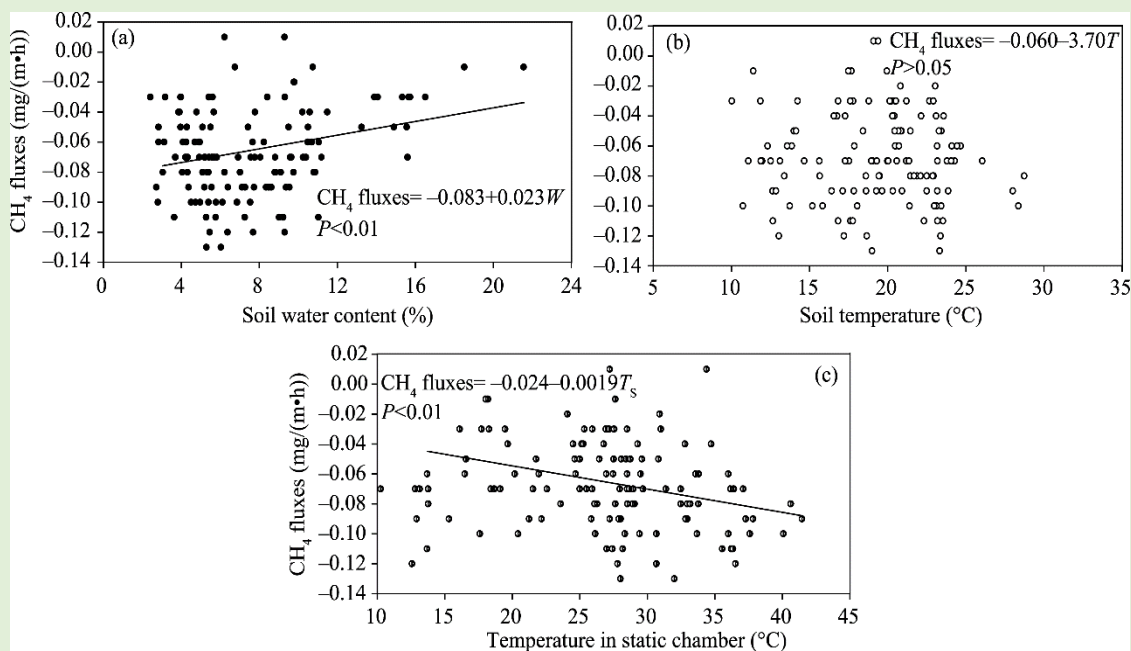


Fig. 7 Relationships of CH_4 fluxes with (a) soil water content, (b) soil temperature at the surface soil (0–10 cm) and (c) air temperature in the static chamber

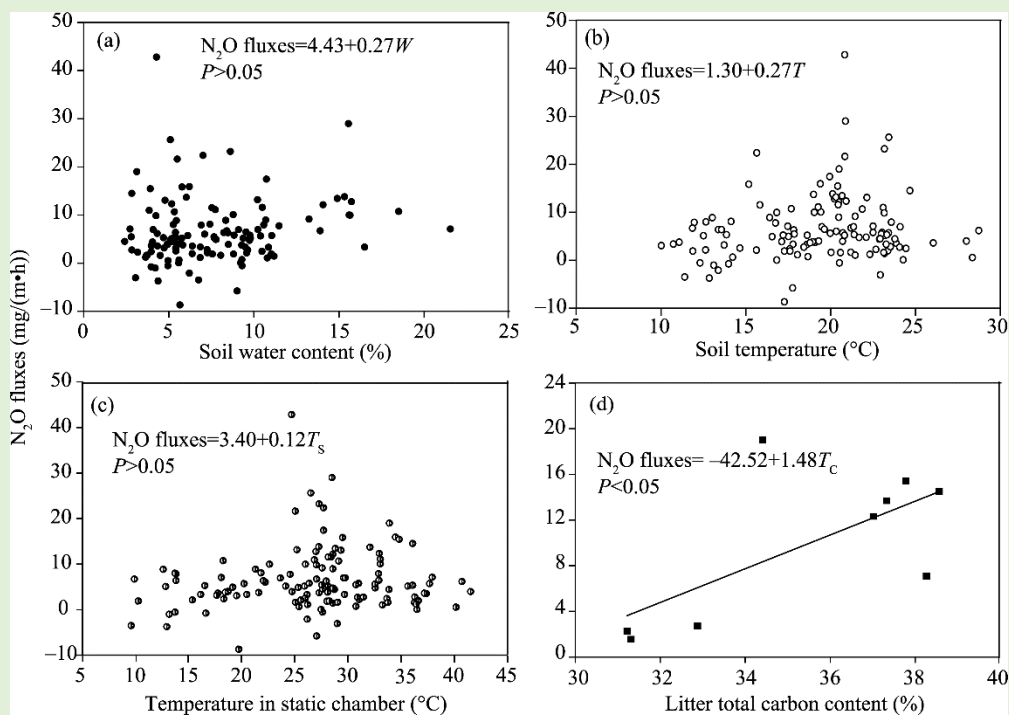


Fig. 8 Relationships of N₂O fluxes with (a) soil water content, (b) soil temperature at the surface soil (0–10 cm), (c) air temperature in the static chamber and (d) litter total carbon content (T_c)

4 Discussion

4.1 Impact of degradation on CO₂ fluxes

Atmospheric CO₂ is assimilated by photosynthesis and released from soil into atmosphere through organic matter decomposition and respiration (Wan and Luo, 2003; Konda et al., 2010). We observed a clear seasonal pattern of CO₂ in typical semi-arid grassland soil of Inner Mongolia during the growing seasons in both 2013 and 2014. The average CO₂ fluxes increased with the degree of grassland degradation during the growing season in 2014 ($P > 0.05$; Fig. 2b). This may confirm previous findings that grassland degradation may increase the GHG emissions from soil to atmosphere (Hirota et al., 2005; Li et al., 2015). Soil biological processes can be stimulated by grassland degradation (Ward et al., 2007) and thus promote CO₂ emissions. At the same time, there were also interactions between vegetation and soil that the vegetation will change the soil physical and chemical properties. Different dominant species may also affect GHG emissions due to differentiated physiological characteristics (Turnbull et al., 2008). In our experimental area, the increased degradation caused the dominant vegetation species to change from *S. grandis* to *S. krylovii*. *S. krylovii* is more suitable for dry conditions and both its aboveground and underground biomass are higher than *S. grandis* (Table 2), which results in the production of more root exudates that promote microbial activity and thus enhance soil respiration and increase soil CO₂ emissions. In our study, the pH, bulk density and nutrients of surface soil increased, during the process of grassland degradation that consistent with previous studies (Li et al., 2013, 2014). Grassland degradation can cause the barrenness of soil nutrients, thus, the changes of the soil nutrients in grasslands with different degree of degradation may produce different degree of negative effects on the C cycle in the typical grassland in Inner Mongolia. Studies have shown that soil properties such as concentrations of total and inorganic N, total C, and bulk density have a significant effect on CO₂ fluxes (Chen et al., 2011a). The results in 2013 were opposite with that in 2014: CO₂ emissions were reduced with increasing degree of degradation, although there was no significant difference between the emissions. In our study, there was no significant relationship

between CO₂ fluxes and NO₃⁻-N. So did NH₄⁺-N, soil organic matter, total N, bulk density and pH. However, we found that CO₂ fluxes positively correlated with surface soil temperature and soil moisture during the growing season (Fig.6). The peak CO₂ flux occurred in late July and August and the lowest flux was observed at the end of the growing season in September in the seasonal pattern following degradation (Fig. 2). These results are consistent with previous studies that the soil temperature and moisture were the major environmental factors that affect the seasonal variation of CO₂ emissions (Liu et al., 2008; Saito et al., 2009; Wu et al., 2010; Li et al., 2015) and that CO₂ emissions were generally higher in the hot-humid season because of more suitable temperature and humidity (Xu et al., 2014; Li et al., 2015). At the end of the growing season in 2013, negative flux was occasionally observed in SD grassland, perhaps partly because the lower temperatures and moisture changes reduced the activity of microorganisms in the soil (Yuste et al., 2007), which changed the balance between soil respiration and CO₂ consumption in the soil (Zhang et al., 2015).

In our study, we found that the major factors affecting soil CO₂ emission were soil moisture and temperature. During the same year, the trends of CO₂ fluxes in grassland with different degradation degrees were similar, although in different years the overall trend was different (Fig. 2a and c). The reason might be due to the differences in soil temperature and moisture conditions between the two years. In addition, the soil CO₂ fluxes were related to the species, quantity and activity of soil microbes. However, we didn't explore the relationships between microorganisms and soil CO₂ fluxes. So further study should be carried out to explore the deeper mechanisms in future.

4.2 Impact of degradation on CH₄ fluxes

CH₄ fluxes observed at the soil surface by the static box method are always the result of CH₄ production and oxidation processes (Conrad, 1996; Butterbach-Bahl and Papen, 2002). The typical semi-arid grassland soils of Inner Mongolia exhibited a sink role for CH₄ from atmosphere. The interplay between CH₄ production and consumption that determined whether soils are a net source or sink for CH₄ (Tate, 2015). In this area, CH₄ consumption was obviously greater than the production, although CH₄ consumption was relatively low (Fig. 3). The average CH₄ uptake rates observed at LD, MD, SD sites for the growing seasons of 2013 and 2014 were 0.067, 0.076, 0.063 mg/(m·h), respectively. The relatively low CH₄ uptake rate is similar to that reported previously on the grazing grassland of the Xilin River Basin during the growing season (0.060±0.044–0.080±0.049 mg/(m·h); Wang et al., 2005). The CH₄ fluxes on different degraded grasslands indicated that the uptake of CH₄ at the MD site was higher than those at the LD site and significantly higher than those at the SD site (Figs. 3b and d). Grassland degradation has a significant effect on CH₄ uptake ($P<0.05$; Table 4). Grazing can change the soil nutrients and its physicochemical properties, and proper grazing is conducive to the accumulation of soil nutrients in grasslands (Clegg, 2006; Gao et al., 2009). Zhou et al. (2008) found that, compared with ungrazing and heavy grazing sites, populations of two types of soil (0–5 cm) methanotrophs were higher in light grazing and moderate grazing sites. In our study, at the MD site, the soil nutrient status, the physicochemical factors of the soil (such as pH, bulk density, soil moisture and temperature) and the populations of soil methanotrophs might have been more conducive to the uptake of CH₄. At the SD site, the grazing rate was heavy, the topsoil was disturbed by animal trampling and decreased the diffusion of CH₄ and oxygen between the atmosphere and soil profile (Liu et al., 2007). During the growing season, the amount of fecal and urine deposition were higher in SD sites, in severe degraded sites the higher fecal decomposition rate may be caused by its production, resulting in the feces-originated CH₄ emissions may be offset more CH₄ uptake than in other sites (Tang et al., 2013). At the same time, the exudates and root debris of plants can act as substrates for methane production (Dou et al., 2016) and massive input of plant root exudates may stimulate the production and emission of CH₄ fluxes (Tong et al., 2012; Li et al., 2013). In our study, the biomass at the MD site was the lowest and the input of root exudates were less than those at the LD and SD sites, resulting in less CH₄ production at the MD site. Some previous studies shown that the activities of methanotrophs were affected by the soil C content,

soil pH and bulk density (von Fische et al., 2007; Konda et al., 2010; Tate, 2015). Whereas in our study, there were no significant relationships between CH₄ fluxes and soil organic matter and between bulk density and pH. However, our results were consistent with other previous studies that soil CH₄ uptake is mainly driven by soil moisture (Shrestha et al., 2004; Chen et al., 2011b; Li et al., 2015) for soils in arid or semi-arid regions. In our study, the CH₄ fluxes was significant positively correlated with soil moisture and the soil moisture at the MD site was higher than those at the LD and SD sites, which may be the main reason that the uptake of CH₄ at the MD site was highest. We observed that the CH₄ fluxes also exhibited a clear seasonal pattern and that the peak CH₄ uptake in the seasonal pattern following degradation occurred in late July and August (Figs. 3a and c)), which was consistent with the peak of soil moisture. No significant correlation between CH₄ fluxes and soil temperature was found in our study (Fig. 7b), which is consistent with Wang et al. (2005) and Dou et al. (2016). However, the CH₄ fluxes were significantly negatively correlated with temperature in the static chambers. This indicates that the clear seasonal pattern of CH₄ uptake was because of the change of soil moisture (Danevčič et al., 2010).

Table 4 Significance of the impacts of month, degradation, year, and their interactions on soil water content, temperature and the fluxes of CO₂, CH₄ and N₂O

	Water content	Soil temperature	CO ₂	CH ₄	N ₂ O
Month (M)	0.039*	0.000**	0.000**	0.001**	0.029*
Degradation (D)	0.000**	0.000**	0.217 ^{ns}	0.012*	0.718 ^{ns}
Year (Y)	0.076 ^{ns}	0.000**	0.703 ^{ns}	0.043*	0.171 ^{ns}
M×D	0.000**	0.000**	0.583 ^{ns}	0.068 ^{ns}	0.961 ^{ns}
M×Y	0.000**	0.000**	0.000**	0.000**	0.039*
D×Y	0.000**	0.000**	0.024*	0.981 ^{ns}	0.407 ^{ns}
M×D×Y	0.000**	0.000**	0.793 ^{ns}	0.377 ^{ns}	0.323 ^{ns}

Note: ** indicates significant impact at $P < 0.01$ level; * indicates significant impact at $P < 0.05$ level; ^{ns}, no significant impact.

4.3 Impact of degradation on N₂O flux

As show in Figure 4, the measured N₂O emission fluxes from steppes during the growing season were usually positive. This suggests that the typical semi-arid steppes of Inner Mongolia is the source of N₂O in the atmosphere. The N₂O emission rates at LD, MD, SD sites for the growing seasons in 2013 and 2014 were 6.60, 7.02 and 5.87 μg/(m·h), respectively. The values were within the range of N₂O growing season rates of 7.07 (±5.19)–8.8 (±6.44) μg/(m·h) (Figs. 4a and c) observed for typical grasslands in Inner Mongolia (Wang et al., 2005).

During the growing season, the N₂O fluxes at different degrees of degradation were inconsistent, as were the average fluxes in 2013 and 2014 (Figs. 4b and d). The grassland degradation had no significant effect on N₂O fluxes (Table 4), possibly because the formation of N₂O is very complicated. N₂O is formed by nitrification and denitrification (Saggar et al., 2004; Pérez et al., 2006). The study of Xu et al. (2003) showed that approximately 64%–88% of the variation of N₂O is produced by nitrification in the Inner Mongolia steppe. At different degraded sites, the process of N₂O formation would be different, which would result in differences in N₂O fluxes. The lack of significant difference between different degrees of degradation may simply due to that the changes of soil substrate and environmental variables caused by grassland degradation were not great enough to lead to a significant changes in abundance and activities of functional microbes. Grazing can stimulate N cycling rate thus has positive effects on nitrification and denitrification but also has negative effect that it can reducing soil moisture in this semiarid environment. The compensation between the positive and negative effects of grazing may attribute to the observed insignificant difference between different degraded grassland soils in N₂O fluxes (Zhong et al., 2014).

In our study, the simultaneous measurements were taken in the different degraded grasslands showed that either soil temperature or moisture had no effect on seasonal N₂O emissions (Fig. 8), which is inconsistent with other studies (Wang et al., 2005; Liu et al., 2008; Chen et al., 2013). This may be because the soil moisture in the study area was mainly concentrated between 4% and

12% (Fig. 8a), which is much lower than that in moist temperate pastures reported by Hyde et al. (2006) and Luo et al. (2008) and in meadow-steppe grassland reported by Zhong et al. (2014), which was not sufficient to have a significant effect on N_2O fluxes. We found that N_2O fluxes were positively correlated with total organic carbon in the litter in our study (Fig. 8d). The litter layer located on the surface soil, can affect the gas diffusion and consumption, while litter can also be decomposed to provide C and N for grassland soil (Li et al., 2004; Wang et al., 2014). The complicated processes of N_2O formation could be controlled by environmental variables, such as soil temperature and moisture (Tang et al., 2006; Liu et al., 2008). At the same time, the formation of N_2O was also controlled by the soil mineral N availability (Maljanen et al., 2012; Benanti et al., 2014), plant biomass (Yamulki et al., 2013; Xu et al., 2014), soil microbial N, and the type and activity of microorganisms (Galbally et al., 2010; Deng et al., 2016). In our study, there were no significant relationships between N_2O fluxes and NO_3^- -N. So did NH_4^+ -N, soil organic matter, total N, bulk density and pH. This might be due to the different effects of NO_3^- -N and NH_4^+ -N on nitrification and denitrification (Firestone and Davidson, 1989; Cardenas et al., 2010). In degraded grassland, the changes of soil properties induced by grazing affected the process of N_2O production (nitrification and denitrification), resulting in insignificant correlation between the N_2O flux and the concentration of mineral N. It was also possible that the N_2O emission from the grassland was relatively low (Wang et al., 2005), the heterogeneity of the grassland was relatively large, and the distribution of soil nutrients was not uniform. Nitrification and denitrification are the main processes of N_2O formation and can be affected by series of intracellular enzymes (such as nitrate reductase, nitrite reductase, nitric oxide reductase) and microorganisms. However, in this study, we mainly explored the total N_2O emissions. So, we studied the effects of grassland degradation on the N_2O production process (nitrification and denitrification), as well as the effects of soil environmental factors and biological factors on its processes to explore the mechanisms of the impacts of different degraded grassland on N_2O fluxes.

5 Conclusions

The typical semi-arid grasslands of Inner Mongolia is the source for atmospheric CO_2 and N_2O and a sink or CH_4 . Compared with CO_2 fluxes, N_2O and CH_4 fluxes were relatively low. Therefore, the exchange of GHG (CO_2 , N_2O and CH_4) fluxes between the grassland soil and the atmosphere may exclusively depend on the net exchange rate of CO_2 in semi-arid grasslands. Grassland degradation significantly influenced CH_4 uptake but had no significant influence on CO_2 and N_2O emissions. Soil moisture and temperature were positively correlated with CO_2 emissions, whereas they had no significant effect on N_2O fluxes. Soil moisture may be the primary driving factor for CH_4 uptake. The main purpose of this study was to explore the dynamic change of GHG fluxes in different degradation degree of grassland during the growing season. However, the processes of GHG formation could be complicated and controlled by many factors, including but not restricted to non-biological factors (environmental factors and so on). In future studies, we will explore the mechanisms of the impacts of degraded grasslands on GHG fluxes by combining various factors such as soil particle size composition, soil microbial C, N content, related enzyme activity and plant species composition.

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